The reproductive biology of the common stingaree *Trygonoptera testacea* (Urolophidae) in eastern Australia

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ABSTRACT

The common stingaree, *Trygonoptera testacea*, is abundant on the continental shelf of eastern Australia but little is known of its ecology and reproduction despite it being a common component of the demersal trawl fishery. Specimens of *T. testacea* were collected from bycatch to investigate the species' reproductive biology. Males were found to mature at a disc width of 22 cm, while females reached sexual maturity at 26 cm disc width. Of all the *T. testacea* examined, 53% of males (n=159) and 16% of females (n=62) were sexually mature. Only the left uterus and ovary were found to be functional in female *T. testacea.* One gravid female carrying two near term embryos was sampled in February 2004. Many other females caught during the same trawl were observed aborting embryos providing a tentative estimate parturition period, which appears to be between the months of February and April. Although further investigation is required to determine if *T. testacea* populations are threatened by fishing pressures, the current study has provided key demographic parameters vital for the design of a management plan for *T. testacea* and similar populations.

Key words batoid; elasmobranch; reproduction; stingaree; Urolophidae

Introduction

In order to implement effective management and conservation measures for a species threatened by fishing pressures it is vital to first determine basic demographic parameters such as size at maturity and fecundity (Conrath and Musick 2002; Kyne and Bennett 2002). Elasmobranch populations of little to no economic importance are often subjected to increased pressure when they form a large component of commercial fishing bycatach (non-targeted catch) (Graham *et al.* 2001). Multi-species fisheries are of particular concern, as elasmobranchs are often a regular component of the bycatch. Such fisheries often continue long after the collapse of the bycatch elasmobranch populations, preventing any chance of recovery (Graham *et al.* 2001).

Stingarees (Urolophidae) are particularly vulnerable to demersal trawling bycatch, since they commonly inhabit flat, sandy habitats where the majority of demersal trawling occurs (Last and Stevens 1994). Furthermore, stingarees have a relatively low fecundity in contrast to bony fish (teleost) with typically 1-4 young per litter (Last and Stevens 1994; White *et al.* 2001; White *et al.* 2002).

The common stingaree, *Trygonoptera testacea* (Müller and Henle 1841), occurs along the coast of eastern Australia (Last and Stevens 1994), where it is frequently caught as bycatch in trawling operations (Kyne *et al.* 2002; pers. obs.). Little biological information is available for this species, despite its "common" status. Last and Stevens (1994) noted that *T. testacea* reaches at least 47 cm total length (TL) and that males attain sexual maturity at approximately 31 cm TL. The size of female *T. testacea* at sexual maturity is unknown.

Given that *T. testacea* is one of the most common species of stingaree in demersal trawl bycatch in eastern Australia, the aim of this study was to investigate key aspects of its reproductive biology, particularly size at sexual maturity, ratio of mature males and females, gestation period and time at parturition.

Materials and methods

Sampling location and regime

All stingarees were collected from bycatch caught off the coast of Newcastle (151º77'E, 32º93'S), New South Wales, Australia (Figure 1). Although the sampling regime was constrained by the schedule of the commercial fishers, each site was only sampled once.

Samples were collected using a trawl net 15 m wide, with 50 mm stretched-mesh in the wings, 45 mm stretched-mesh in the cod end and a 10 mm-thick ground chain at the entrance. Specimens of *T. testacea* were collected from four separate trawls, in August, October and December of 2003, and February 2004. The trawls were in waters ranging from 40 – 50 m deep and trawl speed was approximately 2.5 knots.

Not every stingaree captured was retained for further study, since collection permits allowed for the retention of only 240 specimens. Morphometric data were, however, collected for every stingaree captured. Stingarees were identified to species using Last and Stevens (1994), and the following morphometric data were recorded; sex, total length (TL), disc width (DW), and clasper length

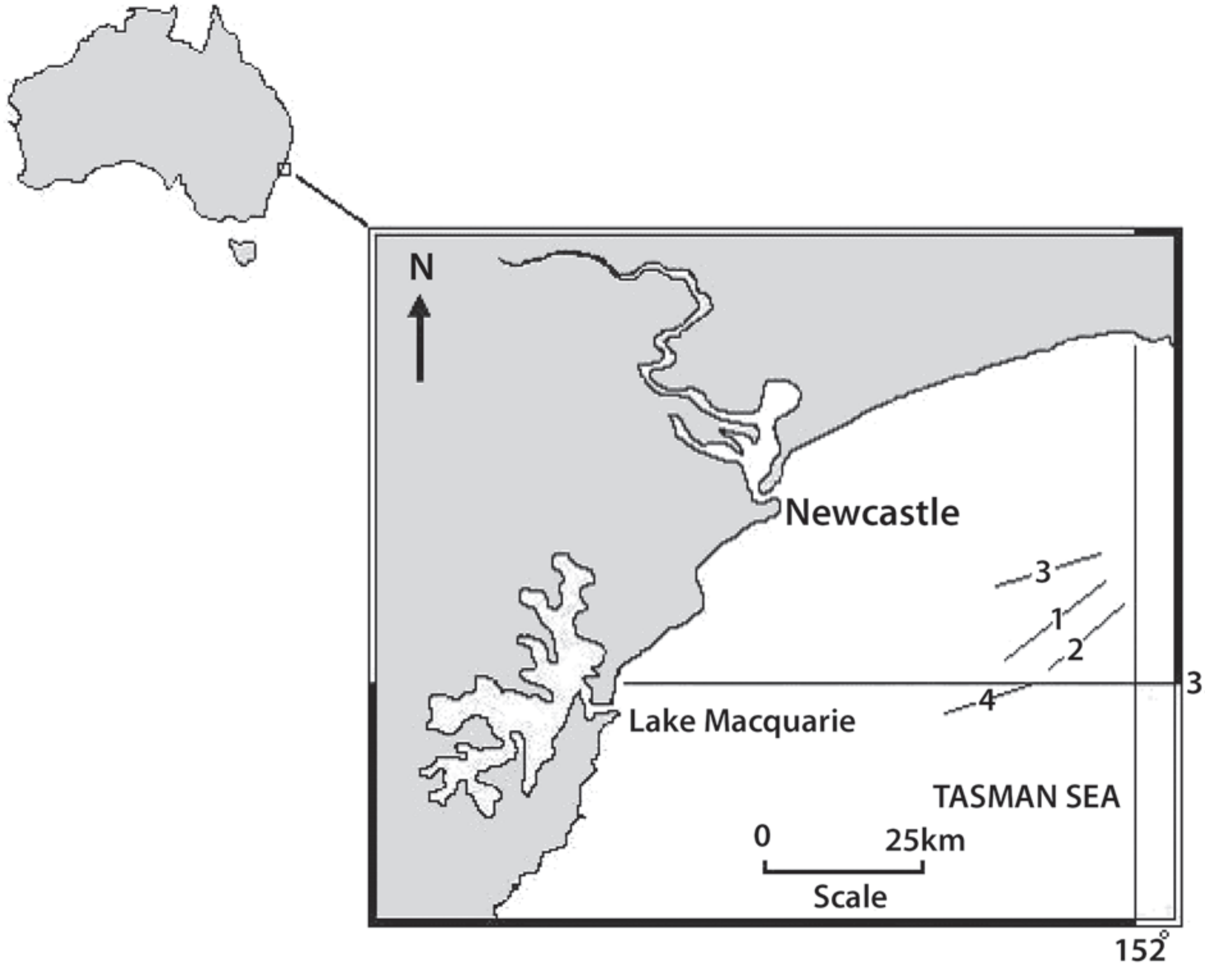

Figure 1. Sample sites off Newcastle, Australia. Lines indicate approximate trawl locations and length. Numbers refer to trawl month. 1) August 2003; 2) October 2003; 3) December 2003; 4) February 2004. Each trawl covered an area of approximately 0.15 km^2.

(CL; for males). Clasper length was measured from the posterior end of the cloacal slit to the tip of the longest clasper, with the specimen lying on its dorsal side and the clasper held parallel to the tail. Specimens in excess of the collection permit conditions were returned to the water after measurement with the remainder of the bycatch.

Reproductive biology of male Trygonoptera testacea

The size of sexual maturity in males was determined using three criteria:

1) The size at which males exhibit an abrupt change in the relationship of CL to DW (Babel 1967; Martin and Calliet 1988; Abdel-Aziz *et al.* 1993; Bridge *et al.* 1998; Conrath and Musick 2002).

2) Male claspers were scored for 'hardness' (Bridge *et al.* 1998; Watson and Smale 1998; Conrath and Musick 2002) and allocated a 'clasper index' (CI) of 1 – 3 by assessing the rigidity of the clasper by hand. An index of 1 was allocated to uncalcified soft claspers (immature), 2 to elongated uncalcified claspers (adolescents), and 3 to elongated calcified claspers (adults). Clasper length was compared to the allocated clasper index in order to assess the accuracy of each technique.

3) The Disc Width (DW) at which the occurrence of coiling in the seminal vesicles in males appears was recorded. Seminal vesicles are straight and undeveloped in immature males. When individuals approach full maturity, the seminal vesicles thicken and become distinctively coiled (Pratt 1979).

Reproductive biology of female Trygonoptera testacea

The following criteria were adapted and modified from several studies (Natanson and Cailliet 1986; Smith and Merriner 1986; Martin and Cailliet 1988; Lenanton *et al.* 1990; Abdel-Aziz 1993; White *et al.* 2001) to recognise three stages of sexual maturity for females.

1) Immature – Ovaries undeveloped and without vitellogenic activity (i.e. no yolked ova, only undeveloped white ova). Uterus thin, strap-like and indistinct from the oviduct.

2) Adolescent – Ovaries not fully developed, ova beginning to enlarge and accumulate yolk.

3) Mature – Large yellow yolked ova present in the ovary, and/or uteri expanded and fully differentiated from the oviducts.

Both the left and right uteri of female *T. testacea* were removed and weighed separately, excluding any eggs and/or embryos that were present. The weight of each uterus was then calculated as a proportion of the total body weight and plotted against DW. This method was compared with the above three maturity criteria to assess whether it could be confidently used to determine female sexual maturity. Fecundity was determined by counting embryos in the uteri of gravid females. Any embryos present were removed from the uterus, weighed, measured for TL and DW, and their sex noted.

Results

Reproductive biology of male Trygonoptera testacea

A total of 159 male *T. testacea* were examined, with the smallest having a DW of 13.5 cm and a TL of 21.6 cm and the largest individual having a DW of 30 cm and TL of 49.3 cm. Of the males examined, 53% were determined to be sexually mature. The smallest sexually mature male in the sample population had a DW of 19.8 cm and a TL of 31.6 cm, and the largest immature male had a DW of 23.1 and TL of 36.8 cm, indicating a size overlap between immature and mature specimens.

The three indices used to determine size of sexual maturity in males – CL, CI, and the coiling of the seminal vesicles – were all strongly related to each other (Table 1). Both CL as a proportion of DW and CI indicated that the approximate size ranges for the stages of sexual maturity were as follows: juveniles had DW of 13 – 18.5 cm; adolescents had DW of 17.5 – 23 cm; and fully mature adults had DW of 19.8 – 30 cm (Figure 2).

The relationship between CL and DW was positive and sigmoidal in form (Figure 2). Clasper development could be broken into three general stages: (a) Small immature individuals are characterised by slow clasper development, with CL approximately 9% of disc width. (b) Adolescent males exhibit a period of rapid clasper development, when CL increases from 9% to approximately 20 – 21% of the DW. (c) Mature adults possess fully developed claspers, with the ratio of CL to DW remaining at about 19% once adulthood is reached (Figure 2).

The coiling of the seminal vesicles was used only to determine if a male was immature or mature, and not to identify adolescents. The seminal vesicles of immature male *T. testacea* were thin and uncoiled. As maturity approached, coiling became more apparent, and increased as individuals reached full maturity. These data indicate that immature individuals ranged in sizes between 13.5 – 23.1 cm DW, while fully mature individuals in this study had a DW of 20.3 – 30 cm (Table 1).

Table 1. Size ranges of male *Trygonoptera testacea* at each stage of maturity as determined by three maturity indices: Clasper length as a proportion of disc width (DW); clasper hardness; and presence of coiling of the seminal vesicles.

Stage of Maturity	Size Ranges for each Male Sexual Maturity Index		
	Clasper Length	Clasper Hardness	Seminal Vesicle Coiling
Juvenile	13.5-19.0 cm DW	13.5-18.4 cm DW	13.5-23.1 cm DW
Adolescent	18.2-23.2 cm DW	17.3-23.2 cm DW	
Adult	19.8-30.0 cm DW	19.8-30.0 cm DW	20.3-30.0 cm DW

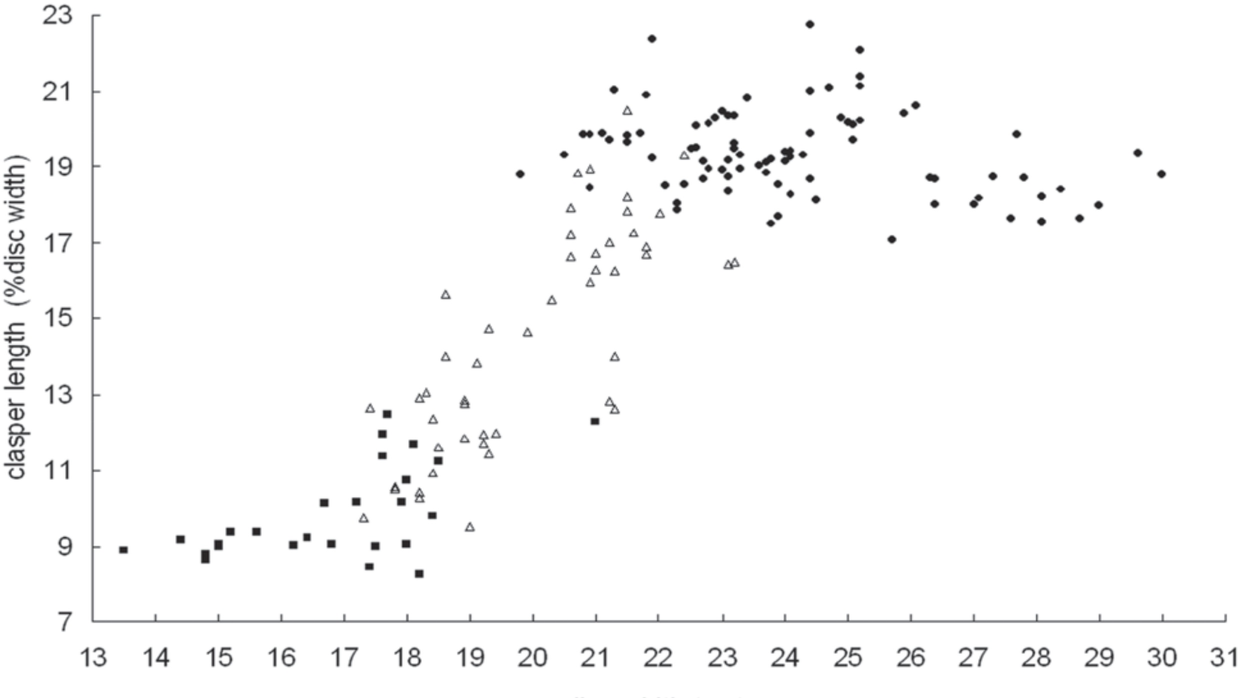

Figure 2. Relationship between clasper length (expressed as a percentage of disc width) and disc width and its association with the clasper hardness index. ■, CI=1 - immature males; Δ, CI=2 - adolescent males; ●, CI=3 - mature males.

Reproductive biology of female Trygonoptera testacea

A total of 62 female *T. testeacea* were examined, with the smallest having a DW of 14.3 cm and TL of 21.9 cm and the largest individual having a DW of 31.3 cm and TL of 51.6 cm. Of the females examined, 16% were determined to be sexually mature. The smallest sexually mature female in the sample population had a DW of 25.7 cm and TL of 40.5 cm and the largest immature female had a DW of 25.9 cm and TL of 42.7 cm.

Mature ova were not observed in any of the right ovaries of female *T. testacea* and the right uterus remained undeveloped. Therefore only the weight of the left uterus as a percentage of total body weight was plotted against DW (Figure 3). The relationship showed an abrupt increase in the relative weight of the left uterus starting when females reached a DW of approximately 23 – 24 cm, and plateauing at a DW of approximately 26 cm. This appears to be an effective measure of assessing female reproductive stages.

Ovaries of females with DW ≤ 20 cm contained only microscopic ova that were white and translucent with diameters of approximately 0.3 – 1.0 mm. Females with DW approximately 21 - 23 cm contained undeveloped ova that were beginning to accumulate yolk (turn yellow) and increase with size. Only ovaries from females with DW > 23 cm contained developed yolked ova. Discrete size classes of ova were readily discernable within the left ovaries of mature individuals, regardless of the time of capture. 2-4 size classes were observed in mature individuals ranging from microscopic white ova to developed yolked ova. The size of the largest ovum observed was 10.8mm in August, 10.3 mm in September, 16.5 mm in December, and 25.3 mm in February.

Only one gravid female was observed in the entire study, with two near-term embryos. This female was caught in February 2004 and had a DW of 26.7 cm and a TL of 43.8 cm. Two male embryos with DW of 8.5 cm and 9.0 cm, each weighing approximately 20 g, were present in the left uterus. The embryos had absorbed their yolk sac and trophonemata (uterine villi) were observed entering the spiracles and mouths of the embryos, characteristics of near-term stingaree embryos (Babel 1967). Many aborted embryos were observed in the February 2004 trawl, although some of these may have been from other species of stingaree that were also captured. No females collected between August and December 2003 appeared to be gravid and no aborted embryos were observed in trawls during this time.

Discussion

Stingarees are demersal, relatively slow swimmers and are born with disc widths of approximately 80 mm (Babel 1967; Last and Stevens 1994; Platell *et al.* 1998; White *et al.* 2002). Since the mesh size of the cod end of the trawl net used in sampling was 45 mm, trawling would have presumably collected the entire size range of the stingarees present in the area covered. The largest specimen collected (a female) was 51.6 cm in total length, an increase on the previous maximum reported

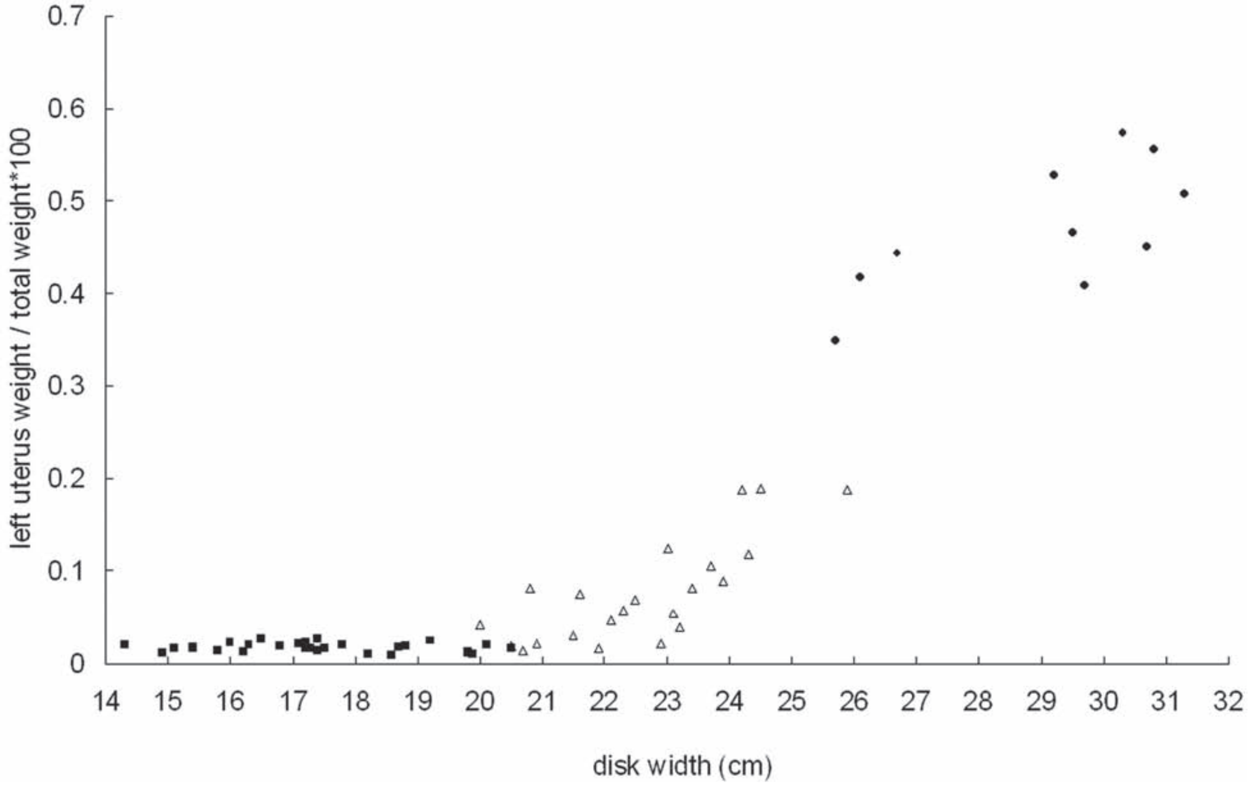

Figure 3. Relationship between left uterus weight (expressed as a proportion of total body weight) and total body weight in female *Trygonoptera testacea.* ■, immature females; Δ, adolescent females; ●, mature females as described by the three maturity criteria.

length of 47 cm for *T. testacea* (Last and Stevens 1994). Unfortunately the size measurements provided by Last and Stevens (1994) were not expressed in terms of disc width (DW) which is the more conventional method used in measuring batoids (e.g. Babel 1967; Martin and Cailliet 1988; Snelson *et al.* 1988; Johnson and Snelson 1996; Platell *et al.* 1998; White *et al.* 2002). Batoids are often observed with incomplete or missing tails, presumably due to predator interactions, therefore length is not a very reliable measure of their reproductive stage.

Reproductive biology of male Trygonoptera testacea

The three indicators of reproductive biology for male *T. testacea* utilised in this study (clasper length, clasper hardness and seminal vesicle coiling) were all strongly consistent (Table 1), suggesting that any one of these measures could be used to assess male maturity in future studies.

The data indicated that 50% of males reach sexual maturity at a disc width of approximately 22 cm and a total length of 35 cm. This value is somewhat larger than the reported size by Last and Stevens' (1994) of approximately 31 cm total length.

Reproductive biology of female Trygonoptera testacea

Only 16% of all female *T. testacea* captured over the entire sampling period were sexually mature. There is a general lack of data concerning the ratios of mature and immature stingarees, but White *et al.* (2001) found that the proportion of mature females of *Urolophus lobatus* fluctuated throughout the year between approximately 20-60%. It is hard to know whether the abundance of sexually mature females sampled during the present study was within 'normal' levels for *T. testacea*, but it was notably lower than the 53% of mature males sampled. It is important to consider that the present study may have sampled soon after parturition, resulting in a large proportion of newborn juvenile rays. It is also possible that larger females may exist in greater abundances in un-sampled areas if segregation by size occurs, as it does in some other species of batoids including *Urolophus halleri* (Babel 1967), *Dasyayis sabina* (Snelson and Williams 1981) and *Myliobatis californica* (Grey *et al.* 1997). Unfortunately stratified and widespread sampling was not possible due to reliance on commercial fishing operations for samples. However, if the present study accurately reflects the population structure of *T. testacea*, the low proportion of sexually mature females caught could be of concern, since it may suggest a high fishing pressure on the population.

The present study found evidence of gravid females only in early February 2004. *T. testacea* is able to give birth to at least 2 young at a time, which is consistent with other *Trygonoptera* species such as *T. personata* and *T. mucosa* which have litters of one or two (White *et al.* 2002).

Many near-term embryonic stingarees were discovered throughout the catch from the February 2004 trawl. A number of female *T. testacea* were also observed actively aborting live embryos on the trawler deck, suggesting there were other pregnant individuals during this period. Also, during dissection of some mature female *T. testacea*, embryonic yolk was discovered in the vicinity of the cloaca, as well as in the large flaccid left uterus. Aborting is commonly reported in studies where stingarees are caught in demersal trawls (Babel 1967; Edwards 1980; White *et al.* 2001; Kyne *et al.* 2002; White *et al.* 2002). It was not possible to identify the aborted embryos to a species level, because another co-occurring species, *Urolophus. sp.* A, was also aborting embryos at a similar stage of development and of a similar appearance (Last and Stephens 1994). The aborted embryos from both species were combined with the entire catch from the trawl, so it was not possible to confidently state which species they had originated from, hindering an accurate assessment of factors such as embryonic sex ratios, percentage of gravid females and reproductive potential.

As no evidence of diapause (a temporary suspension in the development of fertilised eggs or young embryos within the uterus) was observed in this study, and only one gravid female was collected, it is not possible at present to predict the exact period of gestation or parturition time of *T. testacea*. Other species of Australian *Trygonoptera* stingarees - *T. personata* and *T. mucosa* - show a trend towards giving birth around April (White *et al.* 2002), and the embryos observed in the current study were close to term as they had almost completely absorbed their yolk sacs, therefore a tentative estimate of the most likely time for *T. testacea* parturition appears to be between the months of February and April.

In general, due to the low economic value of bycatch species, the evaluation of their sustainability is hampered by a lack of biological information, since funding for research is often limited to studies of commercially important species. Without an in-depth understanding of the reproductive biology of bycatch species it is not possible to assess anthropogenic impacts on their populations and implement conservation measures to ensure their sustainability. Even without comprehensive data, this study suggests there are some preliminary indications that *T. testacea* might be threatened by current fishing practices, since it has high susceptibility to capture in multispecies trawling and as a result, abort foetuses. Also, the success of a population in which recruitment is directly related to the number of sexually reproducing females is dependent on low mortality of juveniles in order to maintain the size of the reproducing portion of the population (Simpfendorfer and Milward 1993). Aborting undeveloped embryos upon capture adds another level of complexity to the effects of demersal trawling on stingarees, since even if the mother survives the trawl and is returned to the water, another generation is lost.

This study represents the first step in gathering the necessary data to assess the conservation status of *T. testacea* in eastern Australia. More detailed information on fecundity and reproductive cycles, as well as information on age, growth and mortality, are still required for comprehensive demographic studies. This in turn, will need to be combined with continued monitoring of catch rates of juvenile and adult *T. testacea*, as well as length-frequency distributions of the entire population, to develop a sustainable management plan for this species.

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APPENDIX I

Common Stingaree *trygonoptera testacea*.
Photo, J. van den Broek.